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Polyamide profiles of porcine milk and of intestinal tissue of pigs during suckling

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Summary — Previous studies have suggested that luminal polyamines can directly influence intestinal differentiation of neonatal rats. The present investigation has demonstrated the presence of high levels of polyamines in porcine milk and in the intestinal tissues of suckling pigs. The quantities of polyamines in sow's milk sampled between wk 1 and 8 of lactation were determined using high performance liquid chromatography (HPLC). The concentration of milk spermidine (SPD) remained constant over the first 3 to 4 wk of lactation but increased 4-fold between wk 4 and 7. Neither putrescine nor spermine (SPN) were detected in any of the milk samples. During intestinal development the mucosal SPD/SPN ratio was elevated between wk 1 and 3 and wk 5 and 7. The latter period of increase corresponded with the surge in milk SPD concentration. It is suggested that milk SPD is taken up from the intestinal lumen and is involved in potentiating intestinal differentiation during the latter part of the suckling period.

polyamine / milk / intestine / disaccharidase / piglet

Résumé — Les profils de polyamines du lait de truie et du tissu intestinal de porcelet allaité. *Des études précédentes ont suggéré l'influence des polyamines sur la différenciation intestinale au cours de la vie fœtale du rat. Ce travail démontre la présence d'un niveau important en polyamines dans le lait de truie et dans l'intestin grêle de porcelet. La concentration en polyamines du lait de truie échantillonné entre la première et la huitième semaine de lactation a été déterminée par chromatographie liquide haute performance. La concentration de spermidine (SPD) du lait reste constante pendant les 4 premières semaines de lactation, puis elle quadruple entre la quatrième et la septième semaine. La putrescine et la spermine (SPN) n'ont pas été détectées dans les échantillons de lait. Pendant la phase de développement intestinal, le rapport de mucosal SPD/SPN augmente entre la première et la troisième semaine, ainsi qu'entre la cinquième et la septière semaine. Cette dernière période d'augmentation correspond a une élévation de la concentration de SPD dans le lait. Il est suggéré que la SPD du lait est prélevée de la lumière intestinale et contribue potentiellement à la différenciation intestinale pendant la dernière période d'allaitement.*

polyamine / lait / intestin / disaccharidase / porcelet

* Correspondence and reprints.

INTRODUCTION

Polyamines are ubiquitous in both prokaryotic and eukaryotic cells and have been shown to facilitate cellular proliferation and differentiation. The biosynthetic enzymes involved in intestinal mucosal polyamine synthesis and the polyamine content of the mucosa show patterns of ontogenic change that can be correlated with those of mucosal disaccharidases in the developing rat (Luk *et al,* 1980a). Furthermore, the post-natal increase in mucosal enzymes can be delayed by the administration of difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, the rate limiting enzyme in polyamine biosynthesis (Luk *et al,* 1980b).

Biosynthesis of polyamines, particularly spermidine, is markedly enhanced in the mammary gland during pregnancy and lactation (Russel and McVicker, 1972) and they have been detected in the milk of a number of mammalian species (Sanguansermsri *et al,* 1974; Brosnan and Yu-Wan, 1985). The aim of the present study was to determine the levels of polyamines in porcine milk during lactation and to describe the ontogenic changes in intestinal tissue polyamine levels in suckling pigs. The results are discussed with regard to the possible role of dietary polyamines in the regulation of intestinal development.

MATERIALS AND METHODS

Milk collection

Lactating sows received a single injection of oxytocin to stimulate milk letdown. Milk samples were obtained by manual expression and stored frozen in sterile containers. On the initial day of the experiment the colostrum was thawed, pooled and maintained in a refrigerated tank with gentle agitation to ensure homogeneity. Milk samples were stored at –20 °C for approximately 3 d prior to preparation for polyamine analysis.

Experimental animals

The progeny for this experiment were derived from 4 sows (Large White x Landrace). Using a combination of concurrent matings and pharmacological manipulations, all sows were farrowed within the same 24-h period. Nine piglets from each sow were taken at birth and housed in sterile incubators (34°C, RH 50%) for the first 36 h of life. Each piglet was fed by gavage approximately 40 ml of standard colostrum every 3 h for 36 h *post-partum.* Animals were suckled for 8 wk on single dams housed in farrowing crates (2.3 x 1.7 x 0.6 m) for the duration of the experiment. To avoid solid feed consumption, piglets were removed from the sows during their feeding periods and any spilled food removed. To maintain hygiene the crates were cleaned and disinfected twice daily. One animal per litter was killed at wk 1; thereafter 2 animals per litter were killed at 3, 5, 7 and 8 weeks *post-partum.*

Post-mortem *procedure*

Piglets were removed from the sows 2 h prior to slaughter. Anaesthesia was induced using halothane/oxygen inhalation. A midline laparotomy was performed to expose the intestines. The stomach and the large intestine were sealed with crocodile clips at the pyloric sphincter and the ileocaecal valve respectively. Divisions were made at 10, 30, 50, 70 and 90% distance from the pylorus to the ileocaecal valve (sites 1–5 respectively). Ten-cm lengths of small intestine were taken at sites 1–5, opened along the mesentery, rinsed with 0.14 M KCl, frozen immediately in liquid nitrogen and stored at –20 °C for a maximum period of 3 wk prior to analyses.

Polyamine analysis

Polyamines were extracted from 5 ml of milk and from the mucosa obtained from 10-cm

lengths of small intestine (SI) (1–2 g wet wt) using 20 ml of 10% perchloric acid. Polyamines were determined by the high-performance liquid chromatography (HPLC) procedure of Seiler and Knodgen (1980) using a Phillips analytical gradient system and an Altex Ultrasphere IP column (5 μ ODS). The internal standard used was 1,7-diaminoheptane dihydrochloride.

Small intestine differentiation indices

Ornithine decarboxylase (ODC) activity was determined on homogenates preparated from triplicate 2-cm lengths of intestine from each of the 5 intestinal sites according to the method of Russel and Snyder (1968). Preparation of mucosal extracts, dilutions and methods for determination of maltase (EC 3.2.1.3) were as described by Kelly *et al* (1990). The substrate concentrations and the incubation conditions were as described by Kidder and Manners (1980). Protein, RNA and DNA were determined using the methods described by Bradford (1976), Sneider (1957) and Greer *et al* (1985).

Statistical analysis

The data obtained from this study was subjected to an analysis of variance using the Genstat package.

RESULTS

Milk polyamines

The concentration of spermidine (μmol/l) remained constant over the first 3 wk of lactation, increased 4-fold by wk 7 and decreased by wk 8 (fig 1). Putrescine and

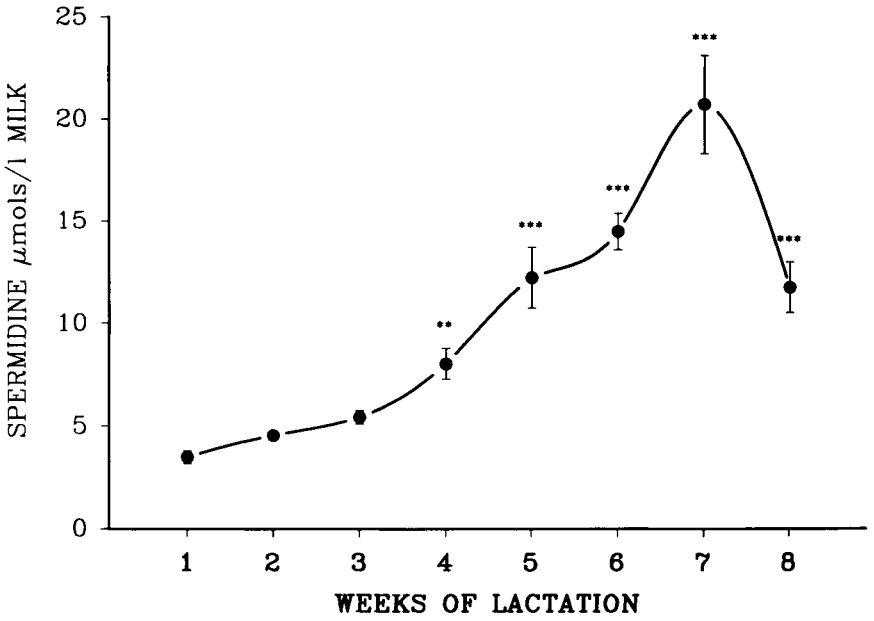

Fig 1. Milk spermidine concentration (μmol/l) (mean ± SEM of 3 milk samples per wk obtained from 7 sows during the first 8 wk of lactation). Statistical significance is expressed relative to wk 1 values. ** = $P < 0.01$; *** = $P < 0.001$.

spermine were not detected in the milk samples.

Intestinal polyamines

Putrescine levels in the intestinal tissue were variable and detected concentrations in the range of 0.04–0.40 μmol/per 10-cm length of small intestine (SI). Putrescine was detected at sites 1 and 2 in approximately 80% of animals but in more distal intestinal sites was detectable in only 40% of animals. There was no significant age effect on putrescine levels and the detailed data are not presented.

The mean spermidine (SPD) concentration (μmol/10 cm SI) of the 5 intestinal sites significantly ($P < 0.001$) increased between wks 1–7 and then decreased at wk 8 (table I). In contrast, when SPD was expressed per g mucosal protein, the highest value was obtained at wk 1 and then the levels significantly decreased at wk 3 ($P < 0.01$) and at wk 5 ($P < 0.001$). However, at wk 7 and 8 a small but conspicuous increase in SPD/g protein was noted (values at wk 8 were 15% higher than the 5-wk values).

Spermine (SPN) concentration (μmol/10 cm SI) increased significantly ($P < 0.001$)

Table I. Polyamine content, mucosal protein, DNA and RNA content and enzyme activity (mean of 5 sites) of the small intestine of suckled pigs at 1, 3, 5, 7 and 8 weeks *post-partum*.

	Weeks post-partum					*SED*	*Statistical significance*
	1	*3*	*5*	*7*	*8*		
Spermidine (SPD)							
μmol/10 cm SI	2.52	3.03	4.39	5.49	4.81	0.482	***
μmol/g protein	22.12	16.92	14.15	15.17	16.25	2.055	***
Spermine (SPN)							
μmol/10 cm SI	1.67	1.89	5.36	5.61	5.31	0.353	***
μmol/g protein	14.66	10.56	17.28	15.50	17.92	2.041	***
SPD/SPN	1.51	1.60	0.82	0.98	0.91	0.101	***
Protein mg/10 cm SI	113.9	179.0	310.2	361.9	296.30	28.22	***
DNA							
mg/10 cm SI	3.29	10.72	14.35	17.36	16.38	18.88	***
μg/mg protein	28.89	59.88	46.26	47.97	55.28	9.88	***
RNA							
mg/10 cm SI	7.57	11.66	28.32	31.07	24.60	18.90	***
μg/mg protein	66.46	65.14	91.30	85.85	83.02	7.21	***
Maltase (μmol/min 10 cm SI)	0.19	0.12	3.99	5.37	5.92	0.558	***

SED, standard error of the difference; ***, $P < 0.001$.

between wk 1 and 5 and remained high for the remainder of the experimental period (table I). SPN expressd per g protein was significantly lower at wk 3 than at the other *post-partum* weeks. The ratio SPD/SPN was high at wk 1 and 3 and was significantly ($P < 0.001$) lower during subsequent weeks (table I). The *post-partum* decline in the SPD SPN ratio was evident at all 5 intestinal sites (fig 2). Proportional differences in SPD and SPN levels gave rise to a secondary increase in SPD/SPN ratio between wk 5–7 which just failed to attain statistical significance. However, this effect was significant ($P < 0.05$) at sites 1, 2 and 5 (fig 2).

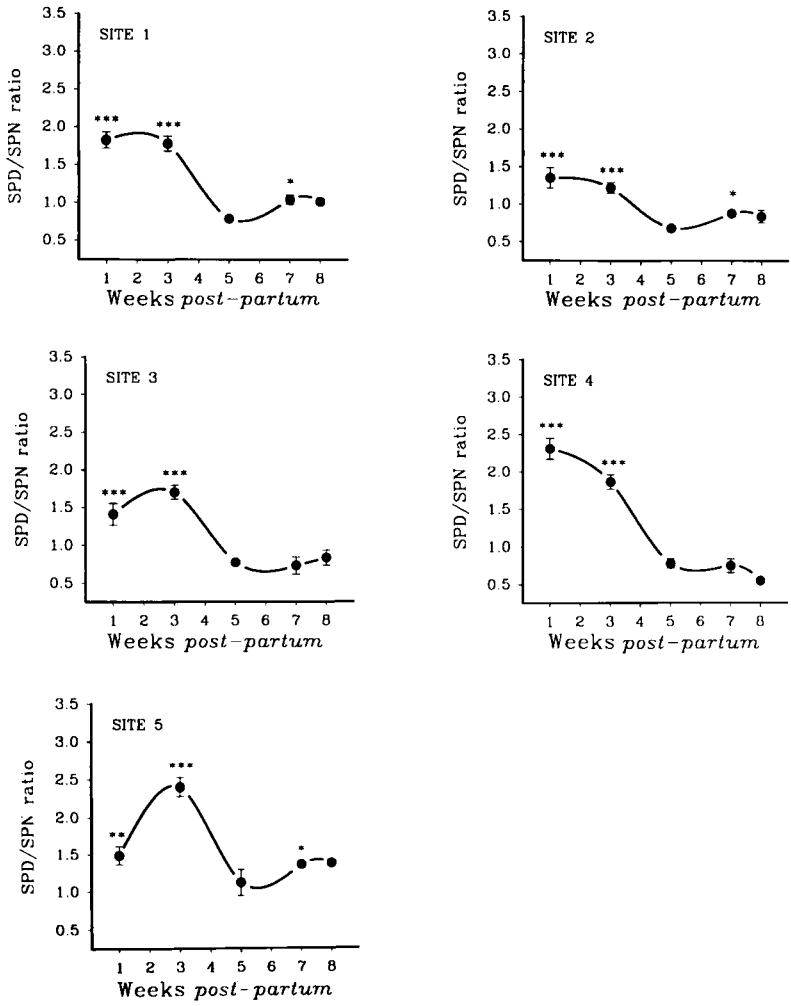

Fig 2. Mucosal spermidine/spermine ratio (mean ± SEM at 5 intestinal sites) of suckled pigs from 1–8 wk *post-partum*. Statistical significance is expressed relative to wk 5 data. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Small intestine differentiation indices

Ornithine decarboxylase (ODC) activities ranged from 1.5–30 nmol/h/g protein or were below the levels of detection. No significant age effect was observed in ODC activity and detailed data have not been presented. There were no significant time x site interactions for any of the other parameters measured and hence the data presented are the mean values for the 5 intestinal sites. Protein and RNA content (mg/10 cm SI) increased significantly ($P < 0.001$) between wk 1–5 (table I). These increases were sustained until wk 7, but the values were slightly lower at week 8. DNA levels (mg/10 cm SI) showed a similar increase during the early *post-partum* period, although the most significant rise occurred between wk 1–3. The early increases in DNA and RNA were also evident when values were expressed per mg protein. Observed trends in the ontogenic changes of maltase activity was constant irrespective of whether the basis of comparison was per unit protein or per unit length of small intestine. Maltase (μmol/min/10 cm SI) was low at 1 and 3 wk and then increased greatly between wk 3–8.

DISCUSSION AND CONCLUSION

The results presented clearly indicate that changes in polyamine levels occur in porcine milk during lactation and in the intestinal tissues of pigs during early post-natal life or during suckling. Polyamine content of milk appears to vary characteristically from one species to another. In the present study the concentration of SPD in porcine milk was found to range from 4–21 μmol/l (≈ 20–100 nmol/g) milk during lactation. This level is comparable to that of rat milk (40 nmol/g milk) but greater than that in human milk (4 nmol/g milk) (Sanguansermsri *et al,* 1974; Brosnan and Yu-Wan, 1985). In the current study, SPN was not detected in porcine milk although it was previously detected in the studies on rat and human milk. In the rat SPD was the predominant polyamine but the converse was true in human milk. In the current study the concentration of SPD in milk was found to increase ≈ 4-fold between wk 4–7 of lactation. This is in agreement with the report of Sanguansermsri *et al* (1974) who described dramatic increases in human breast milk polyamines in the 5th week of lactation. Such changes in SPD concentration during lactation may merely reflect fluctuations in the overall synthetic activity of the mammary gland, but in view of recent studies in the rat (Dufour *et al,* 1988; Bardocz *et al,* 1990) it is plausible that milk polyamines are biologically significant to the developing intestine.

The intestine, like other mammalian tissues and cells, requires polyamines for proliferation and differentiation (Luk *et al,* 1980b; Pegg, 1986). This is the first report of intestinal tissue polyamine levels in neonatal pigs and the data are in good agreement with those published for the neonatal rat (Dufour *et al,* 1988). Intestinal tissue polyamines originate from several sources including *de novo* synthesis, translocation from other organs, diet (Bardocz *et al,* 1990) and luminal bacteria (Osbourne and Seidel, 1989). In the current study, enhanced tissue polyamine levels are likely to be of endogenous and dietary origin since intestinal microbial populations do not change dramatically during suckling (McAllistair *et al,* 1979).

Putrescine, unlike the other polyamines, was detected at relatively low levels in ≈ 40–80% of suckling pigs depending on the sampling site of the intestine. This is likely to be partially related to the sensitivity of technique and equipment, but may also be related to the rapid catabolic breakdown

and translocation of putrescine to other sites relative to SPD and SPN (Bardocz *et al,* 1990). Furthermore, the low levels of both putrescine and ODC in the intestinal tissue might suggest that *de novo* synthesis is not the main source of polyamines during intestinal maturation, although proof of this would require more acute experimentation.

The levels of intestinal tissue SPD and SPN expressed on a protein basis were found to be high at wk 1 relative to the values determined at the other *post-partum* weeks. This is consistent with the findings of Bardocz *et al* (1990) who have shown that during gut hypertrophy, polyamines (particularly SPD) accumulate in the tissue prior to DNA and protein synthesis. Beyond wk 1 the *post-partum* levels of SPD and SPN expressed on a protein basis were essentially constant. In contrast, SPD and SPN concentrations expressed on a unit length basis increased steadily between wk 1–7 concomitant to the increases in mucosal DNA per unit length. Clearly, where conditions or treatments affect cell proliferation and organ growth, analysis of polyamine data expressed on unit length basis is the more sensitive indicator of biological change.

In the current study, high SPD/SPN ratios at wk 1 and 3 *post-partum* were coincident with a period of heightened cellular proliferation, as evidenced by the increase in mucosal DNA levels during this period. The SPD/SPN then decreased at wk 5. This is agreement with the observations of Janne *et al* (1978) who pointed out that high SPD/SPN ratios are generally correlated with rapid cell proliferation and that this ratio decreases with maturity.

In this study, a secondary increase in intestinal SPD/SPN occurred between wk 5–7 and was coincident with the surge in SPD levels in milk. It has been recently shown that enterocytes from the small intestine of the rat contain an energy-dependent transport system for uptake of luminal polyamines (Kumagai *et al,* 1989). However, Bardocz *et al* (1990) have recently suggested that luminal uptake of polyamines relies to a large extent on passive diffusion and that uptake is limited only by dietary supply. Oral administration of polyamines to rat pups has been shown to result in enhanced concentrations within the intestinal tissue (Dufour *et al,* 1988), implying the existence of similar transport mechanisms for luminal uptake in neonates.

The peak in milk SPD concentration at 7 wk was coincident with the peak in mucosal RNA content (per 10 cm SI). Furthermore, the period of rapid increase (wk 3–7) in milk SPD and tissue SPD (per 10 cm SI) corresponded with the rapid increase in maltase activity. Direct experimental evidence for the role of luminal polyamines in neonatal gut differentiation as distinct from the proliferative effects has been provided by Dufour *et al* (1988) who have shown enzyme induction in response to oral administration of polyamine to suckling rats. It is possible, therefore, that milk SPD can be transported from the intestinal lumen of the suckling pig and may be involved in potentiating aspects of cellular differentiation including the enhanced expression of mucosal RNA and carbohydrase activity.

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